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STUDIES OF VICIA FABA ROOT MERISTEMS IRRADIATED WITH A π^- BEAM

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For some time we have been studying the dosimetric character of π^- beams produced by the Berkeley 184-inch cyclotron.[1-3] Our interest stems from the fact that such a beam, if the intensity were sufficiently high, could have important applications in the radiotherapy of cancer.[1,4] The π^--meson has the property that, at the end of its range, it is captured by a nucleus, causing the nucleus to explode into fragments which contain a high proportion of short-range, heavily ionizing α particles and protons. The dose distribution of such a beam in tissue-equivalent material gives a good ratio between the dose to the tumor and the dose to the intervening healthy tissue, even for deep-lying tumors.

Of even greater importance is the fact that the high LET of the particles in the tumor region might overcome some of the radio-resistance of the tumor if it were anoxic.

The most useful beam available at the cyclotron for our purpose is a 90-MeV π^- beam. At this energy the intensity is a maximum and the background is not excessive. The range of these pions is around 25 cm of tissue, which is long for the therapeutic situation. In studying the effects of the stopping pions, therefore, an appreciable amount of Lucite absorber must be used, which not only attenuates the beam

but also produces loss by divergence of the beam. These factors must be considered in irradiating biological materials. The dosimetry of this radiation presents a number of new problems that have not been entirely solved. Among these, at present, is that the background of electrons and muons in the beam amounts to about 40% of the particles.

In spite of the questions that remain to be answered, it is useful to go ahead and look at some biological effects of pions. We have used the Vicia faba root meristem, since it is a simple system and a great deal of work has been done on the response of these cells to x-rays, γ-rays, and α-particles.

Our technique has been to expose roots in the flat or plateau region and in the peak or star region of the π^--meson "Bragg curve." The Bragg curve for these particles is not the usual Bragg curve that one obtains for a beam of α particles or protons. Because of the formation of stars at the end of the range the usual Bragg curve is augmented by the energy released in the medium by the star fragments. In determining this curve we have used two ionization chambers, one as a monitor and the second as a detector. The ionization in the peak, as measured by our chamber filled with 1 atmos argon and carbon dioxide, must be considered as an approximation, since the fragments frequently have ranges much greater than the chamber so that wall effects are important. One advantage of the biological systems is that the medium receives the true dose from the fragments.

The π^- Beam and Exposure of the Root Meristems

Figure 1 shows the arrangement used for making the beam and irradiating the bean roots. The pions are produced by 732-MeV protons striking a beryllium target. Since the pions are negative they are deflected away from the proton beam and leave the cyclotron tank through an appropriate window. A small quadrupole focusing magnet just inside the main concrete shielding helps to focus the pions. The beam then passes through a channel in the wall into the meson cave. When it reaches the cave the beam contains particles of various momenta and therefore a bending magnet is placed here which selects particles of 180 MeV/c to go into the quadrupole focusing magnet. The currents in the two sections of this focusing magnet can be set to focus the pions and also to control the shape of the beam according to the shape of the biological sample.

Before the roots are irradiated a careful run is made with varying thicknesses of Lucite absorber, from which we find the position of the augmented Bragg peak. (Figure 2 shows this curve taken for the second run to study the abnormal anaphases.) One set of bean roots is then placed in the plateau region and the other set in the peak region of the beam. The whole system is carefully lined up horizontally and vertically (with the aid of short exposures of Polaroid film) so that the root tips are irradiated, both in the peak and in the plateau region, by the full beam.

Figure 3 is a photograph of the bean roots in their water-filled Lucite boxes. In this run the beam passed through 2 in. of Lucite before it struck the plateau box, so that the beam would be uniformly spread out over the root meristems. In this particular case compressed

air was bubbled through the boxes to guarantee good oxygenation. The box with the control roots is not shown in the photograph. The monitor chamber can be seen as well as the shielding and the cooling connections to the quadrupole focusing magnet. For the sake of convenience a Jordan dosimeter was put into the beam as the instantaneous monitor. This dosimeter has the meter connected to the sensitive chamber by a long pipe, and the meter was read by a closed-circuit TV system. Occasionally we maximized the beam by changing the position of the target in the cyclotron on the basis of this meter reading.

The Response of the *Vicia faba* Root Meristem to Irradiation

The *Vicia faba* root meristem has been chosen because it is not only extremely simple and economical, but, more important, it is sensitive to the low doses (100 to 400 R) that must be used. Obtaining even these doses requires about 20 hr of irradiation because of the low dose rate.

This paper reports the percentages of total anaphases that were abnormal at intervals after the end of the irradiation. Also the percentage of cells containing micronuclei has been scored at similar intervals. (the root tips were stained with the orcein "squash" method). And thirdly, the growth rate after irradiation has been measured as a fraction of the control rate.

Of the three methods, the growth-rate test is the simplest. One simply raises a number of control and test roots, irradiates, and compares the average growth per day of the test roots with that of the control roots. In this case the average test increment per day is reported as a fraction of the average control increment for the same day.

The literature on growth-rate tests employing Vicia faba is rather extensive. Of note here is the work by Gray and Scholes[5] with prolonged irradiations. One of the many problems caused by prolonged irradiation is that of recovery. That is, while receiving later phases of the dose, the material may still be recovering from the early phases. This recovery phenomenon or dose-rate dependence Gray and Scholes found to be pronounced for x and γ rays but nonexistent for α particles. For x and γ rays, the growth rate dropped only half as much after chronic low exposure as after an acute dose of the same total amount, but the effect on growth rate was the same for α particles whether the dose was given over 10 min or 24 hr. This is of interest here because, in the plateau, pions produce ionization density much as do electrons. Therefore one should find a greater dose dependence in the plateau than in the peak position.

The primary cytological test reported here is the scoring of abnormal anaphases. For Vicia faba this method is very fast and simple, requiring little interpretation. One of the best uses to which anaphases have been put has been the work on the oxygen effect by Thoday and Read,[6,7] demonstrating that the effect of x rays, but not of α particles, is sensitive to the amount of oxygen dissolved in the water containing the roots. It is hoped that the anaphase technique can demonstrate the same difference for pions, if x rays are substituted for the pions at the plateau and the particles formed at the peak are substituted for the alphas.

In Fig. 4 the word "anaphases" is in quotes because cells that are well into telophase have been included in the scoring. This is simply a count of the number of anaphases containing bridges and fragments expressed as a percentage of the total anaphases seen.

The fragments appearing at anaphase form micronuclei at telophase. These micronuclei remain in the cell for some time. For this paper, the number of meristem cells containing one or more micronuclei has been counted (and expressed as percent). Because micronuclei accumulate to some extent, micronuclei counts have proved convenient during the long fixation period to insure that no peaks in the anaphase percentage have been missed between fixation times.

The cell kinetics during a prolonged pion irradiation are only partly understood. Most important is the fact that, after an as yet unknown period of time, the cells in the meristem, both those irradiated at the peak and those at the plateau, stop dividing. This mitotic inhibition is due to the irradiation, and not to physical shock, since control tips placed nearby, but out of the beam, continue to divide. For this reason, much of the irradiation is carried out with the roots in interphase; it is not until about 10 hr after the end of the irradiation that division begins again. Both roots irradiated in the plateau and at the peak appear to resume division at roughly the same time.

Results

Figure 4 shows the peak and plateau anaphase counts for one experiment. Each point represents at least two root tips. When division begins, both peak- and plateau-irradiated roots show the same percentage abnormality; but approximately the same peak-to-plateau ratio is maintained from 12.5 to 98 hr after the irradiation. This ratio over all the points is 2.2 to 1.0. The dose in the plateau region was about 100 R. A second irradiation at a higher dose (about 350 R) has given a ratio of 2.6 to 1.0.

During the second irradiation, a count of the cells containing one or more micronuclei was taken. This is shown in Fig. 5. The micronuclei present at zero hours have resulted from cells that were in mitosis during the irradiation.

Figure 6 shows the results of two growth-rate experiments. About 15 roots were used in each box for each of these experiments. Six days after the irradiation, the rate from peak irradiation has dropped to about 0.45 and that from the plateau to about 0.75 of the control rate.

All three tests indicate that a significantly greater amount of damage has been received at the peak position.

Although these data leave many questions unanswered, the results are very encouraging. About half of the plateau dose is due to contamination; when this is removed with an electrostatic separator, a much clearer picture of the effects of pions will be possible.

In addition, the heavily ionizing particles in the star, particularly the alphas, are known to overcome the oxygen effect. Of interest, then, will be the irradiations in which the water in the boxes has been wholly or partially deoxygenated.

Acknowledgments

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Footnotes and References

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Figure Captions

Fig. 1. The π^--meson leave the target and are focused by a small quadrupole. They then go into the meson cave and there are deflected by the bending magnet and are focused by a second quadrupole. The shielding around the bending magnet and the second quadrupole helps to keep the background radiation low.

Fig. 2. This curve is an approximation to the dose distribution in Lucite. The roots are exposed in the plateau and in the peak regions of the curve.

Fig. 3. The photograph shows the arrangement of the beans in their boxes and the absorbers. Two monitors are used, an ionization chamber upstream and a Jordan dosimeter downstream. The cooling connections to the quadrupole focusing magnet can also be seen.

Fig. 4. The count of abnormal anaphases for the roots irradiated in the peak and in the plateau and for the control roots as a function of fixation time after irradiation. The average is 2.2:1. A second experiment gave 2.6:1.

Fig. 5. The fraction of cells showing one or more micronuclei as a function of fixation time after irradiation.

Fig. 6. The growth rate per day of roots irradiated in the peak and in the plateau of the Bragg curve compared with control roots as a function of days after irradiation.

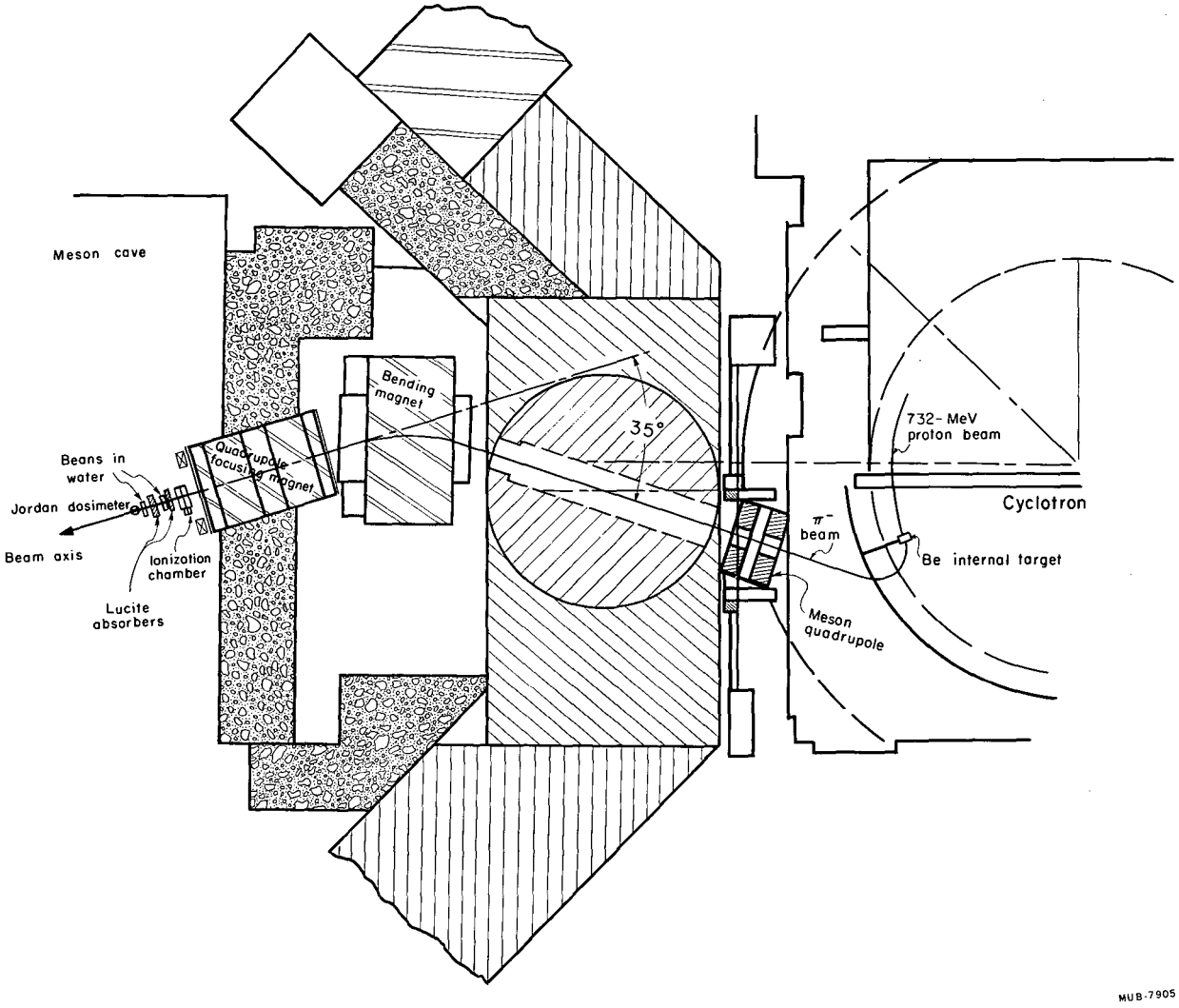
Meson cave
Bending magnet
35°
732-MeV proton beam
Cyclotron
Beans in water
Jordan dosimeter
Beam axis
Ionization chamber
Lucite absorbers
Quadrupole focusing magnet
π⁻ beam
Be internal target
Meson quadrupole
MUB-7905

Fig. 1

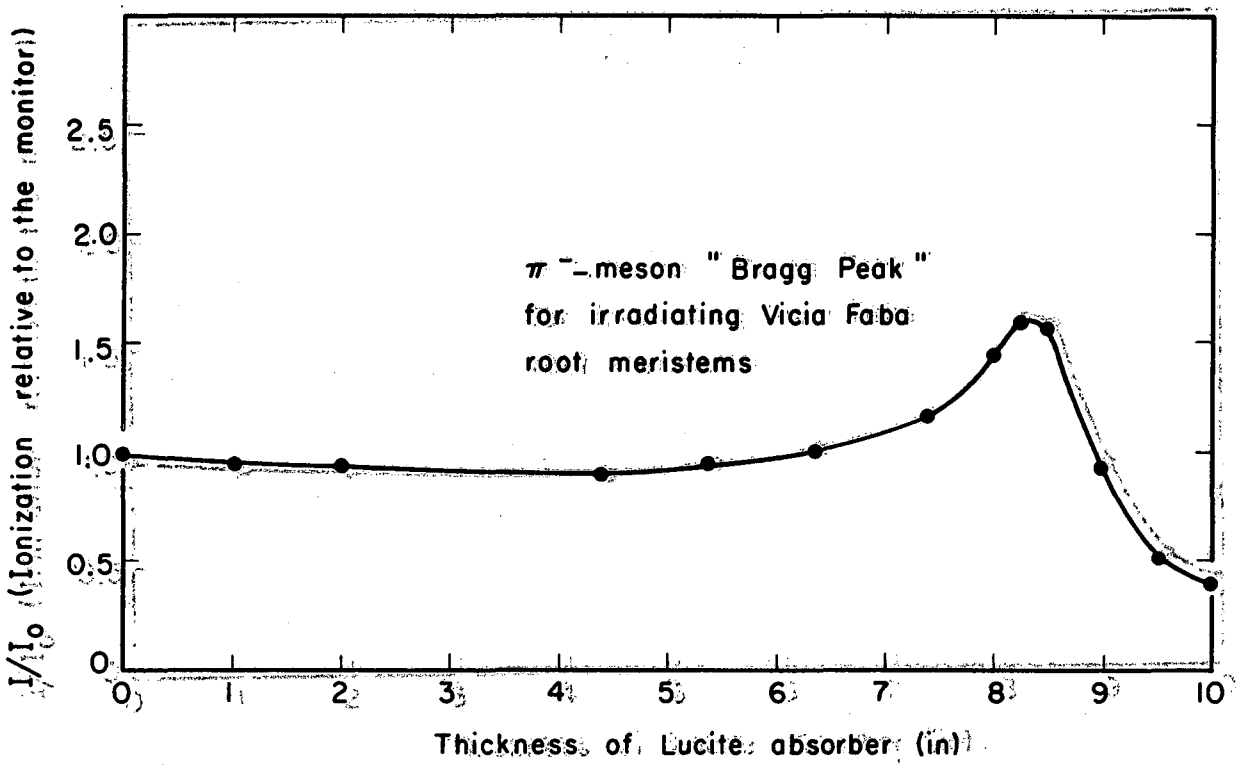

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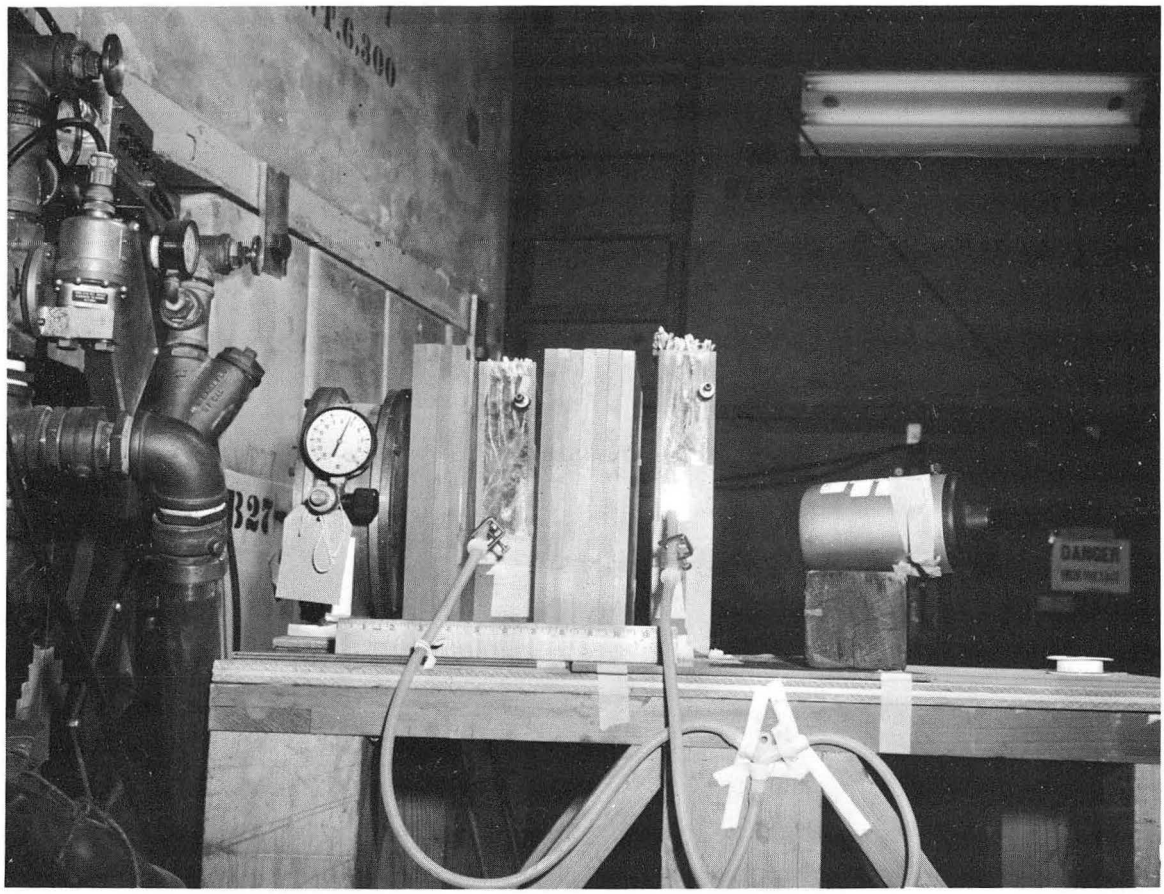
Fig. 2



ZN-5148

Fig. 3

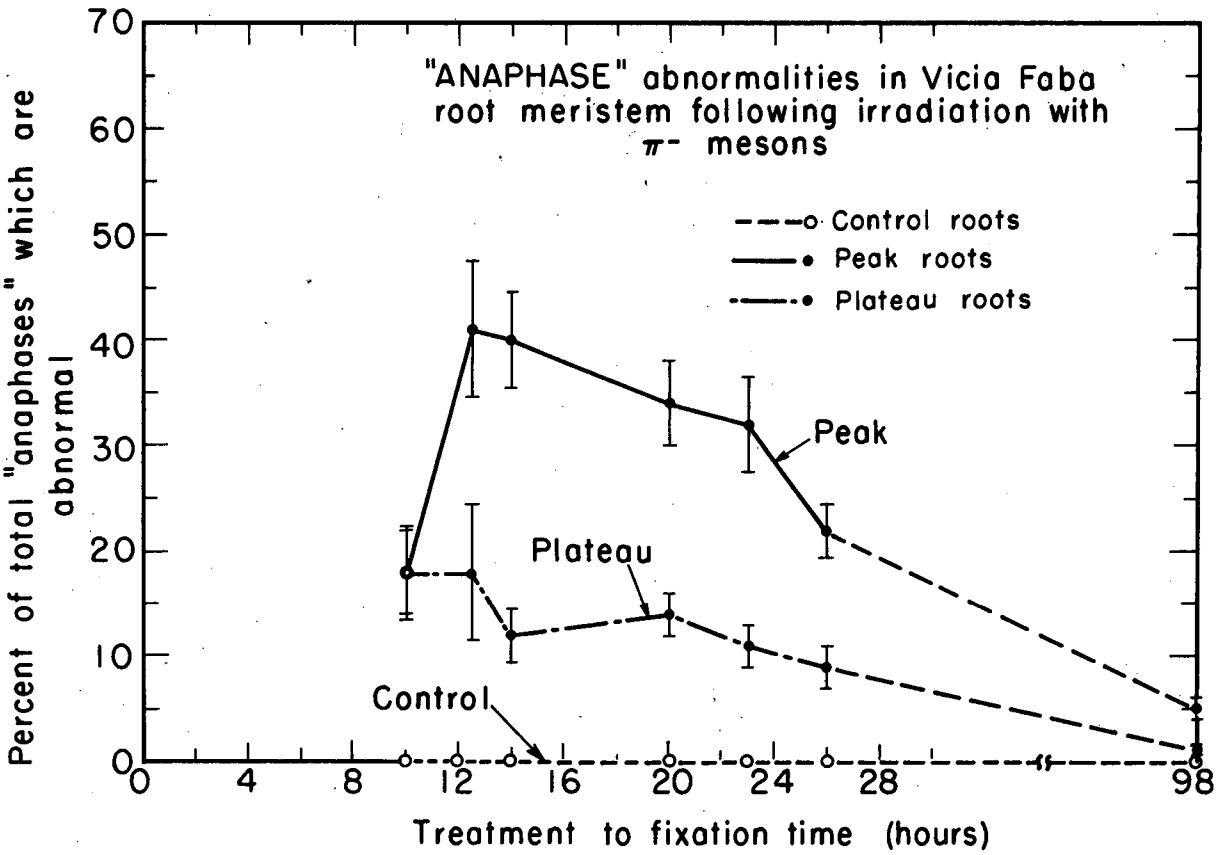


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Fig. 4

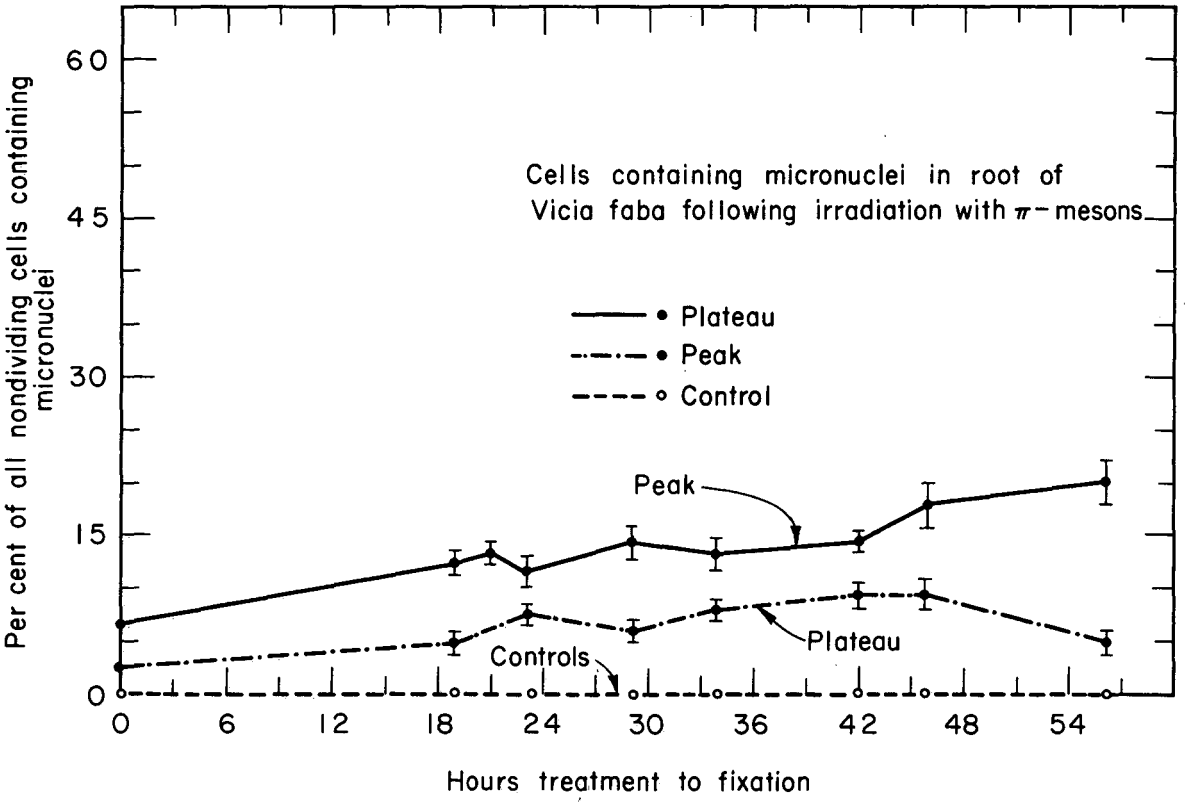

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Fig. 5

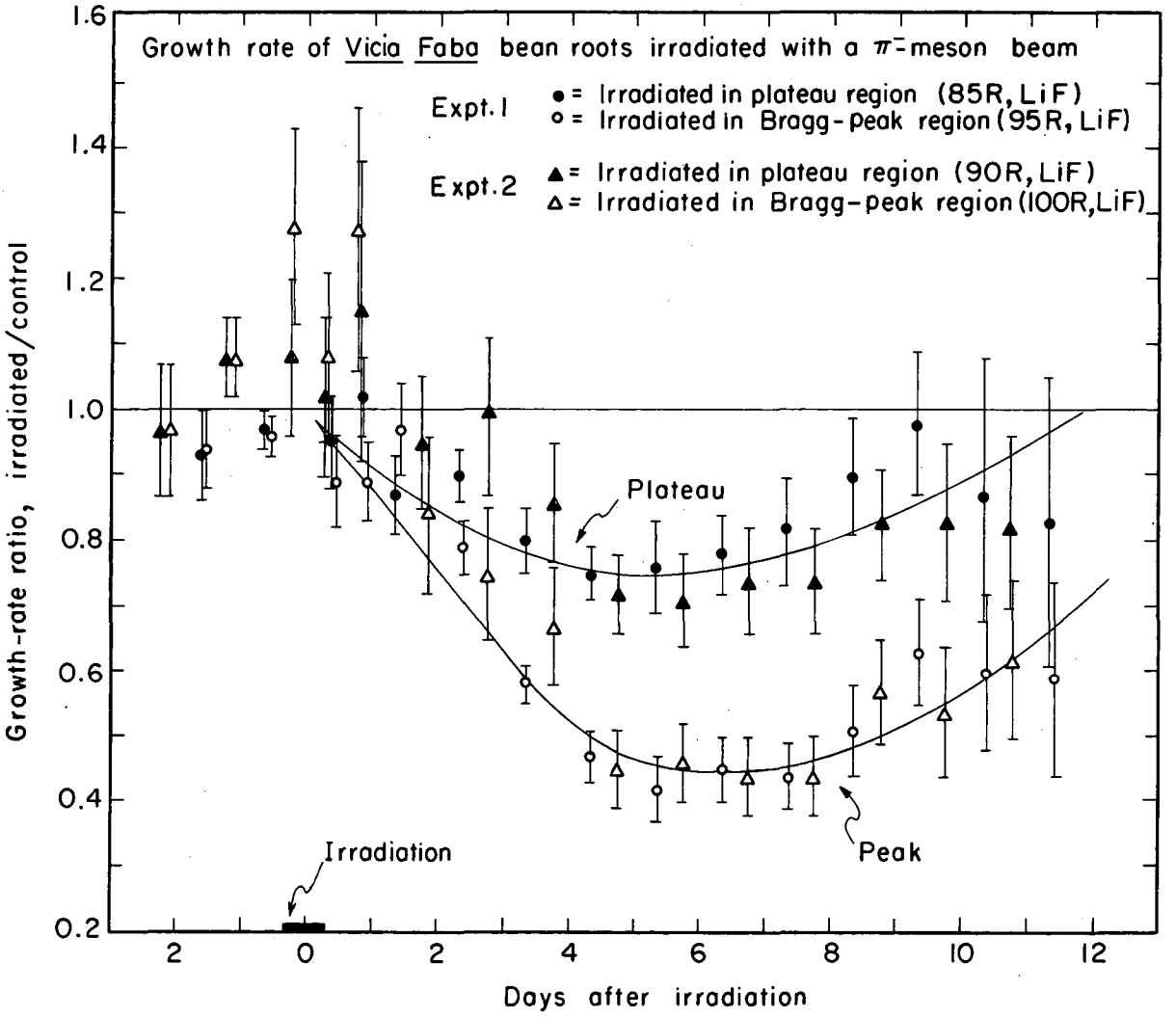

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Fig. 6

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